# C1q/TNF-α–Related Protein 1 (CTRP1) Maintains Blood Pressure Under Dehydration Conditions

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***Rationale:*** **Circulating CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) levels are increased in hypertensive patients compared with those in healthy subjects. Nonetheless, little is known about the molecular and physiological function of CTRP1 in blood pressure (BP) regulation.**

***Objective:*** **To investigate the physiological/pathophysiological role of CTRP1 in BP regulation.**

***Methods and Results:*** **CTRP1 production was increased to maintain normotension under dehydration conditions, and this function was impaired in inducible CTRP1 KO (knockout) mice (CTRP1$^{\Delta CAG}$). The increase in CTRP1 under dehydration conditions was mediated by glucocorticoids, and the antagonist mifepristone prevented the increase in CTRP1 and attenuated BP recovery. Treatment with a synthetic glucocorticoid increased the transcription, translation, and secretion of CTRP1 from skeletal muscle cells. Functionally, CTRP1 increases BP through the stimulation of the AT1R (Ang II [angiotensin II] receptor 1)-Rho (Ras homolog gene family)/ROCK (Rho kinase)–signaling pathway to induce vasoconstriction. CTRP1 promoted AT1R plasma membrane trafficking through phosphorylation of AKT and AKT substrate of 160 kDa (AS160). In addition, the administration of an AT1R blocker, losartan, recovered the hypertensive phenotype of CTRP1 TG (transgenic) mice.**

***Conclusions:*** **For the first time, we provide evidence that CTRP1 contributes to the regulation of BP homeostasis by preventing dehydration-induced hypotension. (*Circ Res*. 2018;123:e5-e19. DOI: 10.1161/CIRCRESAHA.118.312871.)**

**Key Words:** dehydration ■ glucocorticoids ■ hypertension ■ mice ■ vasoconstriction



Adipose tissue is now viewed as an endocrine organ because it can secrete adipose-derived hormones called adipokines, including leptin, TNF-α (tumor necrosis factor-α), and adiponectin.[1] Some of those adipokines influence vasocontractility and regulate blood pressure (BP). For example, both leptin and TNF-α have been shown to affect both vasodilation and vasoconstriction.[2] Adiponectin is a vasodilator that has been shown to increase nitrogen oxide (NO) and inhibits TNF-α production.[3] An adiponectin family paralog, CTRP (C1q/TNF-α–related protein), has recently been identified.[4] CTRP1 (C1q/TNF-α–related protein 1) levels are positively correlated with blood glucose levels and body mass index[5–8]; most studies have focused on the metabolic functions of CTRP1.[9,10] The administration of CTRP1 lowers blood glucose levels in diabetic mice by enhancing AMPK (AMP-activated protein kinase)- and IRS-1 (insulin receptor substrate 1)–mediated energy consumption[11–13]; nevertheless, plasma or serum levels of CTRP1 are increased in patients or animal models of metabolic diseases, such as type II diabetes mellitus and obesity.[8,14,15] These results suggest that high blood glucose levels upregulate CTRP1 levels in an effort to lower blood glucose.[6,7,16] CTRP1 levels are also increased in the serum of hypertensive patients, and CTRP1 stimulates aldosterone production via upregulation of the transcription of cytochrome P450 11β-hydroxylase 2 (*Cyp11b2*), which is the rate-limiting

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## Novelty and Significance

### What Is Known?

- Circulating CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) is specifically expressed in the zona glomerulosa of the adrenal cortex and stimulates aldosterone production.
- CTRP1 is upregulated in both obese and hypertensive patients.

### What New Information Does This Article Contribute?

- CTRP1 is an antihypotensive protein. During dehydration, the production of CTRP1 increases in response to a glucocorticoid to prevent dehydration-induced hypotension.
- CTRP1 regulates blood pressure (BP) by stimulating the Rho (Ras homolog gene family)/ROCK (Rho kinase)–signaling pathway to induce vasoconstriction.

In this study, we show that CTRP1 is an important regulator of BP. Using CTRP1 conditional KO (knockout) and TG (transgenic) mice, we show that the production of CTRP1 is increased on dehydration in response to glucocorticoid and that under these conditions, it prevents the decrease in BP. Moreover, we found that to increase BP, CTRP1 stimulated the membrane trafficking of AT1R (Ang II [angiotensin II] receptor 1) and Rho/ROCK, which leads to vasoconstriction. Because the *Ctrp1* gene emerged at the time of the appearance of terrestrial vertebrates, when they were beginning to be exposed to dry conditions, we surmise that CTRP1 may have evolved to prevent dehydration-induced hypotension. These findings reveal a novel axis of BP regulation under dehydration conditions. Further studies of the CTRP1 signaling axis are likely to provide new understanding of the physiology of dehydration, as well as the pathological states associated with hemorrhage, diarrhea, and diabetes mellitus.

### Nonstandard Abbreviations and Acronyms

| | |
|---|---|
| **AMPK** | AMP-activated protein kinase |
| **Ang II** | angiotensin II |
| **AT1R** | angiotensin II receptor 1 |
| **BP** | blood pressure |
| **CAG** | chicken β-actin promoter/enhancer coupled with the cytomegalovirus enhancer |
| **CD36** | cluster of differentiation 36 |
| **CTRP** | C1q/TNF-α–related protein |
| **CTRP1** | C1q/TNF-α–related protein 1 |
| **DBP** | diastolic blood pressure |
| **Dex** | dexamethasone |
| **ERK** | extracellular signal-related kinase |
| **ET-1** | endothelin-1 |
| **G-CSF** | granulocyte-colony stimulating factor |
| **GAP** | GTPase-activating protein |
| **GEF** | guanine nucleotide exchange factor |
| **GLUT** | glucose transporter |
| **GR** | glucocorticoid receptor |
| **GRE** | glucocorticoid response element |
| **IL-12** | interleukin-12 |
| **IRS-1** | insulin receptor substrate 1 |
| **KO** | knockout |
| **MCP-1** | monocyte chemoattractant protein 1 |
| **Mife** | mifepristone |
| **MLC** | myosin light chain |
| **MLCK** | MLC kinase |
| **MLCP** | MLC phosphatase |
| **MYPT** | myosin phosphate target |
| **MYPT1** | myosin phosphate target subunit 1 |
| **PKCα** | protein kinase Cα |
| **PP1cδ** | protein phosphatase 1, catalytic subunit δ |
| **Rho** | Ras homolog gene family |
| **RhoA** | Ras homolog gene family, member A |
| **ROCK** | Rho kinase |
| **SBP** | systolic blood pressure |
| **sm22α** | smooth muscle protein 22α |
| **TG** | transgenic |
| **TNF-α** | tumor necrosis factor-α |
| **TNFR1** | tumor necrosis factor receptor 1 |
| **Triam** | triamterene |
| **WT** | wild type |

enzyme for aldosterone production.[17] CTRP1 is also considered to be a novel biomarker for coronary artery[18–22] and heart[23] disease because patients with this disease have increased circulating CTRP1 levels. In addition, CTRP1 has been identified as a regulator of vascular function[24–26] and as an antiacute ischemic injury protection in the heart.[27] These accumulating pieces of evidence support the notion that CTRP1 plays an important role in the regulation of cardiovascular function. Nonetheless, no physiological or molecular roles for CTRP1 in blood vessel function or in the regulation of BP have been described.

The regulation of BP is a complex physiological process that relies on the collaboration between the cardiovascular, neural, renal, and endocrine systems.[28] In the endocrine system, the renin-Ang II (angiotensin II) system is the best known hormone cascade for the regulation of BP through the control of blood volume and vasoconstriction.[29] In particular, AT1R (Ang II receptor 1) mediates the Ang II-induced vasoconstriction through the regulation of phosphorylation and dephosphorylation of MLC (myosin light chain), which directly affects the smooth muscle motility.[30] AT1R stimulates GEF (guanine nucleotide exchange factor)-mediated GDP/GTP exchange of Rho (Ras homolog gene family), which activates ROCK (Rho kinase) through the conformational change followed by the inhibitory phosphorylation of MYPT1 (myosin phosphate target subunit 1).[30] MYPT1 composes MLCP (MLC phosphatase) with PP1cδ (protein phosphatase 1, catalytic subunit δ) and a small regulatory subunit (M20).[30] ROCK-mediated MYPT1 phosphorylation inactivates the phosphatase catalytic subunit PP1cδ, leading to sustained MLC phosphorylation. Contrary to MLCP, activatory phosphorylation of MLCK (MLC kinase) phosphorylates MLC. When MLC is phosphorylated, in turn, vasoconstriction is stimulated, and BP is increased.[30] Significantly increased Rho/ROCK activity and MYPT1 phosphorylation is observed

in hypertensive patients, and this supports that the Rho/ROCK-signaling pathway is highly related to the hypertension.[31]

In the present study, the physiological/pathophysiological function of CTRP1 in vascular Rho/ROCK-signaling pathway and BP regulation was elucidated using genetically modified mouse models, including CTRP1 conditional KO (knockout) and TG (transgenic) mice.

## Methods

Detailed descriptions of experimental materials and methods used in this study are provided in the Online Data Supplement. The authors declare that all supporting data are available within the article and its Online Data Supplement.

## Results

### CTRP1 TG Mice Show a Hypertensive Phenotype

We have previously shown that CTRP1 is highly expressed in the zona glomerulosa of the adrenal gland and increases aldosterone production.[17] Thus, to determine whether CTRP1 is involved in BP control in vivo, CTRP1 TG mice were generated using *Ctrp1* gene expression cassette under the control of the CAG (chicken β-actin promoter) as reported previously.[16] CTRP1 expression was significantly increased in various tissues, as well as in serum (Figure 1A and 1B). Not only monomers (≈35–40 kDa) but also multimers of CTRP1 (≈130–170 kDa) were present in the sera of CTRP1 TG mice (Figure 1C).

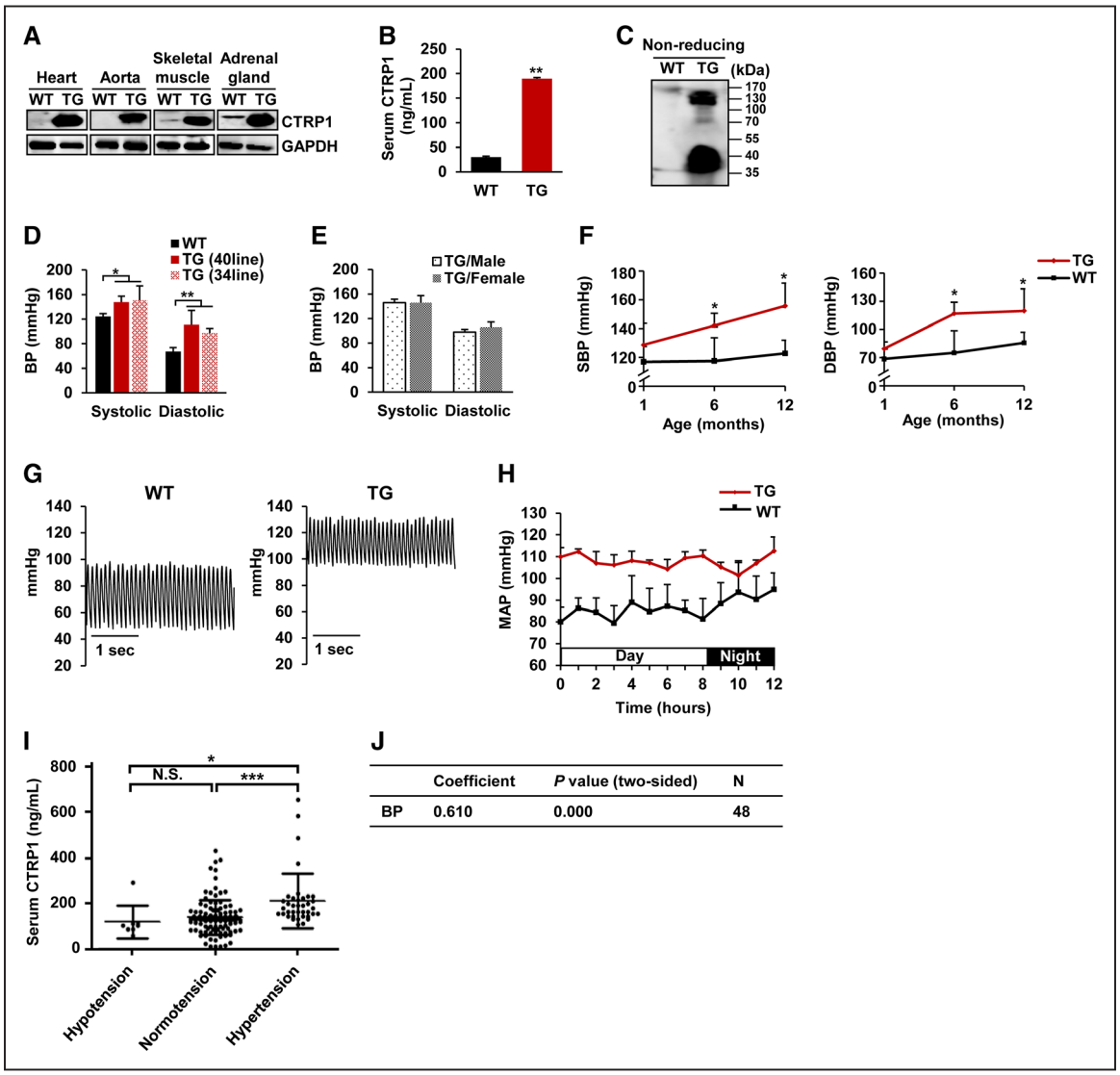


**Figure 1. CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) TG (transgenic) mice showed a hypertensive phenotype. A**, CTRP1 protein levels were analyzed in the indicated tissues from WT (wild type) and CTRP1 TG mice by immunoblotting. GAPDH protein levels were analyzed as a loading control. **B**, CTRP1 levels in sera from WT and CTRP1 TG mice were quantified by ELISA (n=8–12 per group). ***P*<0.01, 2-tailed Student *t* test. **C**, The native forms of serum CTRP1 were analyzed by immunoblotting. **D**, The blood pressure (BP) in 2 different lines of CTRP1 TG (lines 34 and 40), as well as WT mice, was measured using the tail-cuff method (n=6 per group). **P*<0.05, ***P*<0.01, Dunnett test. **E**, BP in male and female CTRP1 TG mice was determined using the tail-cuff method. The male and female mice were age matched (n=4 per group). **F**, Age-dependent BP increases in CTRP1 TG and WT during the period from 1 to 12 mo of age were determined using the tail-cuff method (n=4–6 per group). **P*<0.05 between CTRP1 TG and WT mice, 2-tailed Student *t* test. **G** and **H**, BP in CTRP1 TG and WT mice was measured using radiotelemetry (n=3–4 per group). Representative telemetric recordings of BP (**G**) and mean arterial pressure (MAP) during the day and night (**H**) are shown. **I**, Circulating CTRP1 levels in the sera of newly diagnosed patients with hypotension (n=8), hypertension (n=38), and healthy individuals (normotension, n=119) were measured by ELISA. **P*<0.05, ****P*<0.001, Tukey test. **J**, Pearson correlation coefficient between circulating CTRP1 levels in the sera and BP was calculated. All values are presented as mean±SD. DBP indicates diastolic blood pressure; N.S., no significant difference between the groups indicated by brackets; and SBP, systolic BP.

Next, tail-cuff BP monitoring was conducted to examine whether CTRP1 TG mice had increased BP. Systolic (SBP) and diastolic BP (DBP) were both increased in 2 independent CTRP1 TG lines compared with WT (wild type) mice (Figure 1D). There were no differences in BP in CTRP1 TG mice from either sex (Figure 1E). The significant increase in BP in CTRP1 TG mice began at 6 to 10 months of age (Figure 1F). To further demonstrate the BP changes, CTRP1 TG mice were implanted with radiotelemetry devices, and BP was monitored. BP and mean arterial pressure measured by telemetry were significantly higher in CTRP1 TG mice than in WT mice (Figure 1G and 1H). Circulating CTRP1 levels were also measured in genetic and surgical hypertension models. Circulating CTRP1 levels were highly increased in sera from spontaneous hypertensive rats compared with normotensive Wistar-Kyoto rats (Online Figure IIA), whereas no alternation of circulating CTRP1 levels was observed in the 2-kidney 1-clip hypertensive rats compared with sham-operated normotensive rats (Online Figure IIB and IIC). These results indicate that circulating CTRP1 levels would be differently affected by pathogenic causes of hypertension.

Furthermore, compared with normotensive healthy subjects, newly diagnosed hypertensive patients showed statistically increased circulating CTRP1 levels (Figure 1I), and circulating CTRP1 levels and BP showed the linear correlation each other (Figure 1J).

## Aged CTRP1 TG Mice Show Anatomic and Functional Alterations in the Heart

Next, we examined the effect of CTRP1 overexpression on cardiovascular and renal structures because histological alterations in those BP-related organs could be a cause of the BP increase. No alterations in thoracic aorta thickness were shown in 1-month-old CTRP1 TG mice. However, these significantly increased in 12.6-month-old CTRP1 TG mice compared with age-matched WT mice (Online Figure IIIA). In addition, the left ventricular wall thickness (Online Figure IIIB) and heart weights (Online Figure IIIC) were unaffected in 1-month-old CTRP1 TG mice but were increased in 12.6-month-old CTRP1 TG mice compared with age-matched WT mice. Echocardiography revealed that 12.6-month-old CTRP1 TG mice had impaired cardiac contractility as demonstrated by decreased ejection fractions and left ventricular fractional shortening, and increased end-systolic volumes, left ventricular internal dimension in systole, and interventricular septal thickness at diastole (Figure 2A) without changes in left ventricular end-diastolic volume and left ventricular internal diameter end diastole. These alterations in the cardiovascular apparatus and echocardiographic parameters in aged CTRP1 TG mice are similar to those in cases of patients with chronic hypertension.[32] Furthermore, 12.6-month-old CTRP1 TG mice showed renal hypertrophy (Online Figure IIID) and nephropathy, which included glomerulonephritis, vasculitis, and ectopic accumulation of unidentified vacuoles (Online Figure IIIE). On the contrary, other regulators of BP, including Ang II, aldosterone, electrolytes ($Na^+$, $K^+$, $Cl^-$), urine, and blood volume (Figure 2B through 2E), were not changed in CTRP1 TG mice compared with WT mice. The effect of CTRP1 on cardiac contraction was also examined. Either CTRP1 deficiency or excess conditions did not significantly alter the phosphorylation of molecules involved in cardiac contractility, indicating that CTRP1 would not directly affect to cardiac contraction although CTRP1 could indirectly influence the cardiac function through prolonged BP increase (Online Figure IV).

We also found no circadian variation of BP in CTRP1 TG mice during day or night period (Figure 2F). Moreover, both transcription and translation levels of CTRP1 in various tissues, as well as circulating CTRP1 levels, were not significantly altered in WT mice during the day (Figure 2G through 2I), indicating that CTRP1 is unlikely affected by the daily fluctuation of cortisol. Collectively, these findings indicate that morphological and functional impairments in the cardiovascular and renal tissues are secondary to CTRP1-induced chronic hypertension.

## Dehydration-Induced Glucocorticoid Increased CTRP1 to Prevent BP Drop

Next, we asked which physiological conditions were associated with it. Considering that diabetic patients have highly increased circulating CTRP1 levels,[6–8] circulating CTRP1 levels were measured in streptozotocin-induced type I diabetic mice (Online Figure VA). Circulating CTRP1 levels were markedly increased (Figure 3A), but oral administration of glucose did not significantly further increase circulating CTRP1 levels (Online Figure VB), indicating that the increased circulating CTRP1 levels were not because of hyperglycemia in streptozotocin-injected mice. Because streptozotocin-injected mice show frequent urination, we investigated whether dehydration in the diabetic mice may be a cause of the CTRP1 increase. To test this possibility, the water supply was limited for 2 days to induce dehydration conditions, and circulating CTRP1 levels were measured. As a result, circulating CTRP1 levels were increased by 3-fold on day 2 after water restriction and recovered to normal levels when water supply was restored (Figure 3B). We next hypothesized that increased urination is related with the CTRP1-mediated BP increase. Surprisingly, WT mice remained normotensive and had a normal hematocrit during water restriction (Figure 3C; Online Figure VC), whereas CTRP1 CMV (cytomegalovirus)/β-actin–specific conditional KO mice ($CTRP1^{\Delta CAG}$) were hypotensive and failed to maintain normotension under water-restricted conditions (Figure 3D), indicating that CTRP1 increases BP under dehydration conditions. To further test this hypothesis, WT mice were orally administered various diuretics to induce urine excretion-mediated dehydration. Consistent with the results from water restriction, the diuretic-treated WT mice showed highly increased circulating CTRP1 levels with a normal BP range (Online Figure VD and VE).

Norepinephrine and glucocorticoid hormones prevent BP decreases under dehydration conditions. To determine whether these hormones are associated with the CTRP1 increase under dehydration conditions, mice treated with the diuretic triamterene (Triam) were concurrently treated with β-adrenergic blocker propranolol or GR (glucocorticoid receptor) blocker mifepristone (Mife). Combined treatment with Triam and Mife inhibited the increase in circulating CTRP1

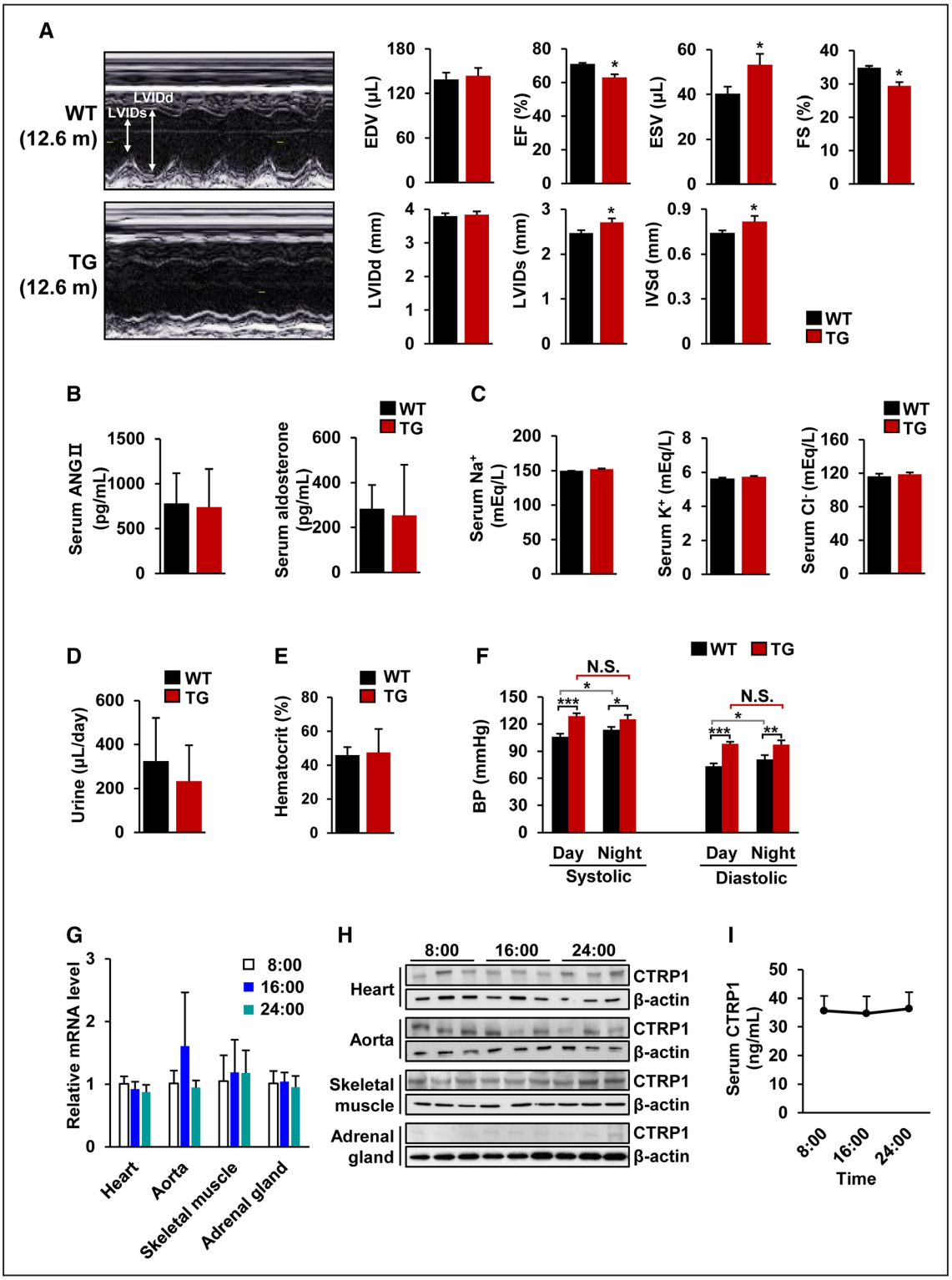


**Figure 2. Aged CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) TG (transgenic) mice show anatomic and functional alterations in the heart. A**, Representative echocardiogram images and echocardiographic parameters from 12.6-mo-old WT (wild type) and CTRP1 TG mice (n=3 per group). *$P$<0.05, 2-tailed Student $t$ test. **B** and **C**, Ang II (angiotensin II) and aldosterone (**B**) and electrolyte (**C**) levels in the sera from WT and CTRP1 TG mice were measured by ELISA or a blood chemistry analyzer (n=6–10 per group). **D**, WT and CTRP1 TG mice were individually housed in metabolic cages, and 24-h urine excretion was recorded (n=8–9 per group). **E**, Anticoagulated whole blood was collected from WT and CTRP1 TG mice by cardiac puncture and the hematocrit measured (n=3 per group). **F**, Blood pressure (BP) in WT and CTRP1 TG mice during the day and night was measured using radiotelemetry (n=3–4 per group). *$P$<0.05, **$P$<0.01, ***$P$<0.001, Tukey test. **G** and **H**, CTRP1 levels in the various tissues indicated were analyzed by qRT-PCR (quantitative real-time polymerase chain reaction) using 18S RNA for normalization (**G**) or by immunoblotting using β-actin for normalization (**H**; n=3 per group). **I**, Levels of CTRP1 in sera from WT mice over time were quantified by ELISA (n=3 per group). For all studies, mice were age matched. All values are presented as mean±SD. EDV indicates end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; FS, fractional shortening; IVSd, interventricular septal thickness at diastole; LVIDd, left ventricular internal diameter end diastole; LVIDs, left ventricular internal diameter end systole; and N.S., no significant difference between the groups indicated by the brackets.

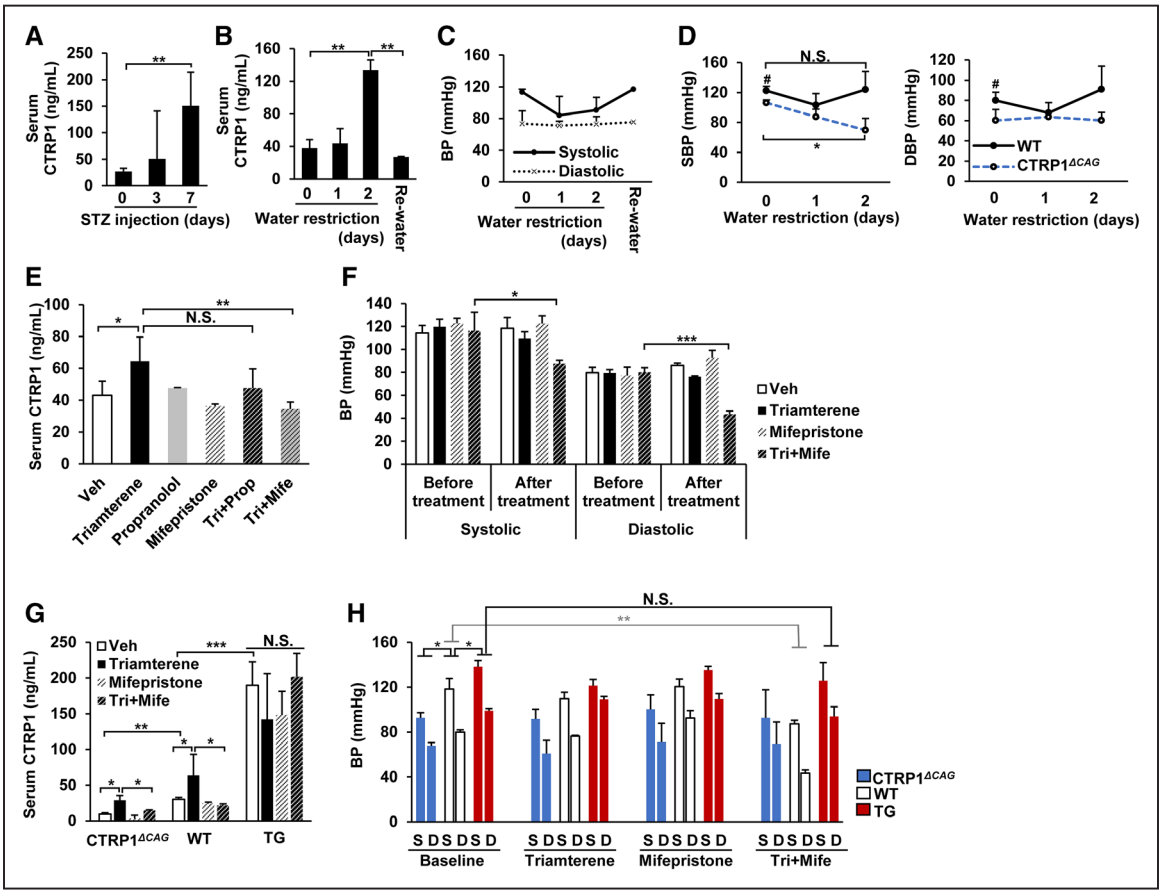


**Figure 3. CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) contributes to glucocorticoid-mediated blood pressure (BP) control under dehydration conditions. A**, CTRP1 levels in sera from WT (wild type) mice injected with streptozotocin (STZ; 120 mg/kg; n=4). ***P*<0.01, Tukey test. **B** and **C**, CTRP1 levels in sera (**B**) and BP (**C**) were determined in water-restricted mice using ELISA and the tail-cuff method, respectively (n=6 per group). ***P*<0.01, Tukey test. **D**, CTRP1$^{\Delta CAG}$ mice were placed in a water-restricted environment, and BP was measured using the tail-cuff method at the indicated times (n=3–4 per group). **P*<0.05, between water restriction day +0 and day +2, Tukey test; #*P*<0.05, between WT and CTRP1$^{\Delta CAG}$ mice, 2-tailed Student *t* test. **E** and **F**, WT mice were pretreated with propranolol (Prop; 10 mg/kg) or mifepristone (Mife; 0.1 mg/kg) 30 min before being orally administered with triamterene (Triam; 60 mg/kg) for 2 d after which CTRP1 levels in the sera (**E**) and BP (**F**) were determined using ELISA and the tail-cuff method, respectively. Water was used as the vehicle control (n=6–8 per group). **P*<0.05, ***P*<0.01, ****P*<0.001, Tukey test. **G** and **H**, CTRP1$^{\Delta CAG}$, WT, and CTRP1 TG (transgenic) mice were pretreated with Mife (0.1 mg/kg) 30 min before being orally administered with Triam (60 mg/kg) for 2 d, after which CTRP1 levels in sera (**G**) and BP (**H**) were determined using ELISA and the tail-cuff method, respectively. Water was used as the vehicle control (n=6–8 per group). **P*<0.05, ***P*<0.01, ****P*<0.001, Tukey test. For all studies, mice were age matched. All values are presented as mean±SD. DBP/D indicates diastolic BP; N.S., no significant difference between the groups indicated by the brackets; and SBP/S, systolic BP.

levels but not with propranolol (Figure 3E). Circulating corticosterone levels were also significantly increased along with circulating CTRP1 levels under the dehydration conditions induced by streptozotocin injection, water restriction, and Triam administration (Online Figure VF through VH). In addition, the combined treatment of Triam with Mife reduced both SBP and DBP along with the CTRP1 decrease (Figure 3F), supporting that increased glucocorticoid signaling is a key mediator of increased CTRP1 under dehydration conditions. To further prove this glucocorticoid-CTRP1 link, BP was monitored in CTRP1 TG mice after combined treatment of Triam with Mife. In CTRP1 TG mice, BP was not affected by the Triam and Mife cotreatment (Figure 3G and 3H). This is likely because of the excessive CTRP1 levels in CTRP1 TG mice. In the case of CTRP1$^{\Delta CAG}$ mice, the basal BP level was decreased because of the low level of CTRP1 (Figure 3H). Collectively, these findings reveal that the glucocorticoid-mediated CTRP1 increase is a crucial factor for maintaining BP during dehydration.

## Dexamethasone Directly Increases CTRP1 in the Skeletal Muscle

The positive regulation of CTRP1 by glucocorticoid was additionally confirmed in electric foot shock-received mice. Circulating CTRP1 and glucocorticoid levels were simultaneously increased under electric foot shock conditions (Online Figure VIA and VIB). Moreover, CTRP1$^{\Delta CAG}$ mice were less sensitive to increase in BP arising from electric foot shock compared with WT mice (Online Figure VIC), suggesting that CTRP1 regulates BP in response to glucocorticoid.

Next, we asked whether glucocorticoid directly increases CTRP1 levels. To address this, WT mice were injected intraperitoneally with dexamethasone (Dex) or were given a restricted water supply. As a result, CTRP1 protein and mRNA expression levels were significantly increased in serum, skeletal muscle, and heart after either Dex treatment or under the dehydration conditions (Figure 4A through 4C). Because CTRP1 levels increased in the skeletal muscle

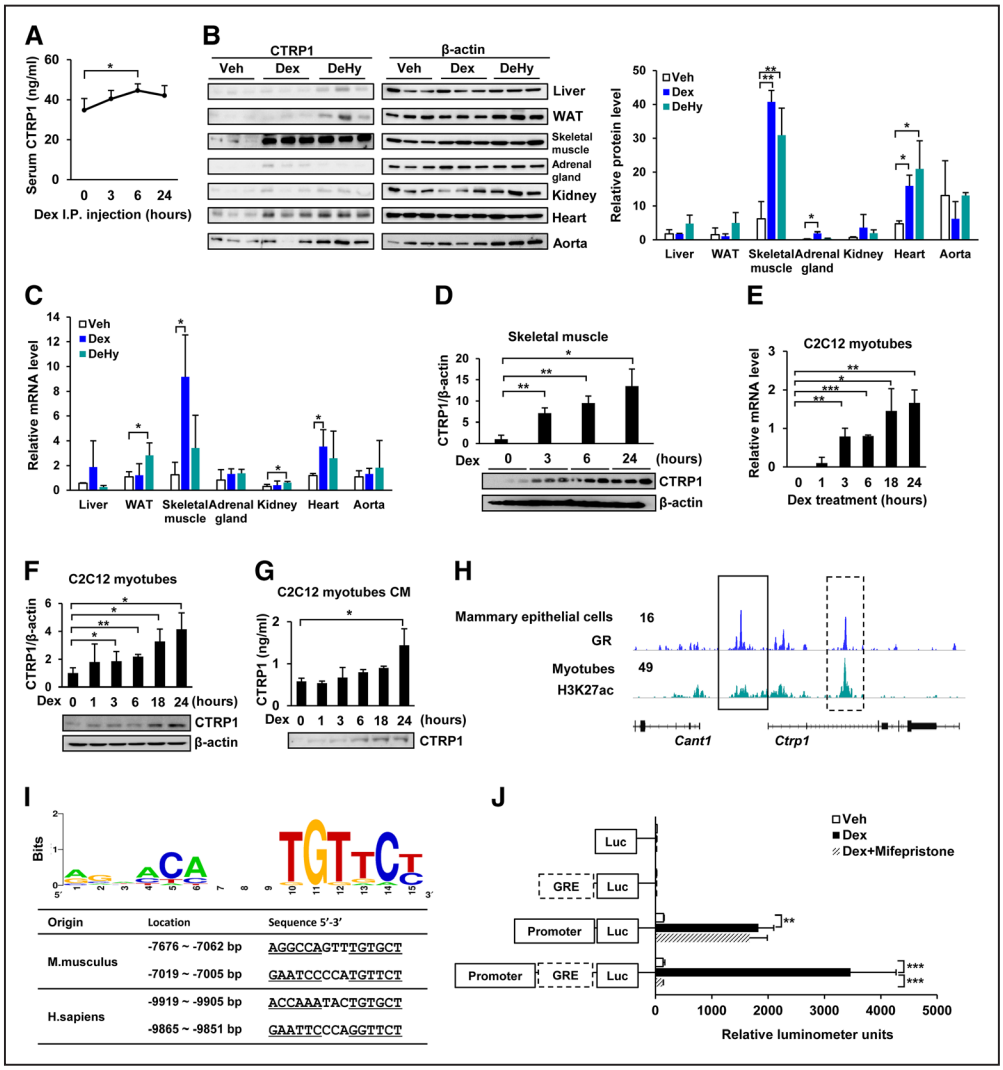


**Figure 4. Dexamethasone (Dex) upregulates CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) expression in the skeletal muscle. A**, Changes in the levels of CTRP1 in sera over time from WT (wild type) mice injected (intraperitoneal [I.P.]) with Dex (10 mg/kg; n=4 per group). *$P$<0.05, Tukey test. **B** and **C**, WT mice were injected (I.P.) with Dex (10 mg/kg) and kept for 6 h, or were dehydrated (DeHy) for 2 d, after which CTRP1 levels in the various tissues indicated were analyzed by immunoblotting using β-actin for normalization (**B**) or by qRT-PCR (quantitative real-time polymerase chain reaction) using 18S RNA for normalization (**C**; n=3–5 per group). *$P$<0.05, **$P$<0.01, Tukey test. **D**, Time course of changes in CTRP1 levels in the skeletal muscle from WT mice injected (I.P.) with Dex (10 mg/kg; n=3 per group) and analyzed by immunoblotting for CTRP1 expression using β-actin for normalization. The immunoblot (**top**) and quantification of the CTRP1/β-actin ratio (**bottom**) are shown. *$P$<0.05, **$P$<0.01, Dunnett test. **E–G**, Fully differentiated C2C12 myotubes were treated with Dex (2 μM) for the indicated times, and the CTRP1 levels were analyzed by qRT-PCR (**E**) and immunoblotting (**F**). Culture media (CM) was collected, and CTRP1 protein levels were analyzed by immunoblotting (**G**, **bottom**) and ELISA (**G**, **top**). β-actin levels and 18S RNA levels were used as loading controls for the immunoblot and the qRT-PCR analysis, respectively. *$P$<0.05, **$P$<0.01, ***$P$<0.001, Dunnett test. **H**, Identification of the GR (glucocorticoid receptor)-binding site in the *Ctrp1* intron region. Genome browser snapshot of GR and H3K27ac at *Ctrp1* locus. The frame with solid line and dashed line refer to the putative promoter region and intronic enhancer co-occupied with GR binding, respectively. **I**, Locations and sequences of the potential glucocorticoid response elements (GREs) in the *Ctrp1* intron region. **J**, The effect of Dex on activity of predictive GRE region. HEK-293T were pretreated with mifepristone (20 μM) 30 min before Dex treatment (2 μM), and firefly luminescence was determined. Mice were age matched for in vivo study. **$P$<0.01, ***$P$<0.001, Tukey test. All values are presented as mean±SD. Luc indicates luciferase; N.S., no significant difference between the groups indicated by the brackets; and Veh, vehicle.

in a time-dependent manner after Dex treatment, the skeletal muscle could be the main tissue that contributed to the CTRP1 increase by glucocorticoid (Figure 4D). To further examine this hypothesis, C2C12 myoblasts cells were differentiated into myotubes and then treated with Dex. Both CTRP1 mRNA and protein levels were found to increase in a time-dependent manner after Dex treatment (Figure 4E and 4F), and furthermore CTRP1 secretion was also upregulated (Figure 4G) by Dex treatment. Additionally, Mife-mediated inhibition of glucocorticoid signaling abolished the CTRP1 increase in the skeletal muscles under dehydration conditions, supporting the hypothesis that the main source of CTRP1 is the skeletal muscles under dehydration conditions in response to glucocorticoid signaling (Online Figure VIIA and VIIB).

We next determined whether GR binds to the promoter region of *Ctrp1* gene and performed ChIP-seq (chromatin immunoprecipitation sequencing) at *Ctrp1* locus. GR occupancy was observed between exon 1 and exon 2 of *Ctrp1* gene. The active enhancer marker H3K27ac coincides exactly with GR binding in this region (Figure 4H). Further analysis of this region revealed that there are 2 potential

glucocorticoid response elements (GREs) within the intron region of *Ctrp1* gene, which showed high homology between mouse and human (71%; Figure 4I; Online Figure VIII). To find out whether the potential GREs are functional, human GRE-containing region was cloned and ligated at the downstream of a putative human *Ctrp1* gene promoter region, and reporter assays were performed. Solely GRE was not enough to increase the luciferase activity by Dex, but the putative promoter GRE showed a significantly increased luciferase activity by Dex, which was strongly suppressed by Mife (Figure 4J). Solely putative promoter was also responsive to dexamethasone but not affected by Mife (Figure 4J). These data support the notion that CTRP1 is directly increased by dehydration-induced glucocorticoid.

## CTRP1 Increases BP Through Vascular Rho/ROCK Activation

Next, to explore the molecular mechanism by which CTRP1 increases BP, we determined whether CTRP1 directly induces aortic contraction because vascular contractility is a crucial in BP upregulation and the development of hypertension. Endothelium-intact thoracic aortas isolated from Sprague Dawley rats were treated with CTRP1, and its contractile effect was monitored for 60 minutes. As a result, CTRP1 increased the contractile force of the aorta 1 to 5 minutes after treatment (Figure 5A), and this effect was dose dependent and comparable with that of 5 μM of Ang II (Figure 5B), implying that CTRP1 increases BP via vasoconstriction.

To identify the downstream signaling pathway by which CTRP1 induces vasoconstriction, MLC phosphorylation was

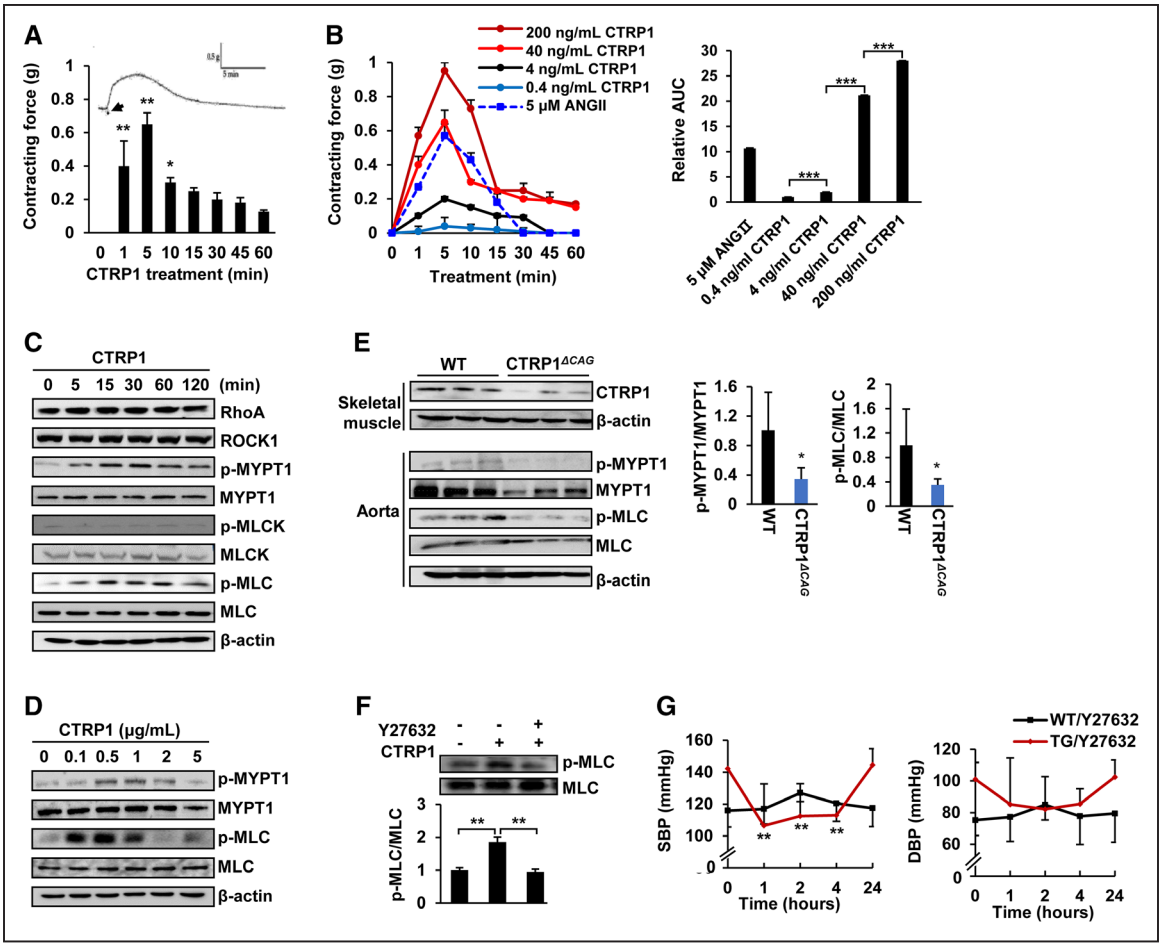


**Figure 5. CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) induces vasoconstriction through the RhoA (Ras homolog gene family, member A)/ROCK (Rho kinase)-signaling pathway. A**, The time course contraction of thoracic aortic rings isolated from Sprague Dawley rats measured after CTRP1 treatment (40 ng/mL). The contractile activity of CTRP1 is shown both as a bar graph (**bottom**) and a physiogram (**top**, inset). The dot in the physiogram (see arrow) indicates the moment of addition of CTRP1. **P*<0.05, ***P*<0.01, Dunnett test. **B**, Dose-dependent contraction of thoracic aortic rings isolated from Sprague Dawley rats in response to CTRP1 treatment (**left**) and quantification of the areas under curve (AUCs; **right**). Ang II (angiotensin II) was used as a positive vasoconstrictor. ****P*<0.001, Tukey test. **C**, The levels of vasoconstriction-related proteins in MOVAS after CTRP1 treatment (1 μg/mL) were analyzed using immunoblotting. β-actin protein levels were used as the loading control. **D**, Dose-dependent changes in the levels of p-MLC and p-MYPT1 in MOVAS 30 min after CTRP1 treatment by immunoblotting. β-actin protein levels were analyzed as a loading control. **E**, Levels of the indicated proteins in the skeletal muscle or aorta from WT (wild type) and CTRP1$^{\Delta CAG}$ mice were analyzed by immunoblotting (**left**) and their ratios with respect to their total levels were quantified (**right**; n=5). **P*<0.05, 2-tailed Student *t* test. **F**, MOVAS were pretreated with the Rho/ROCK pathway inhibitor Y-27362 (30 μM) 30 min before CTRP1 treatment (1 μg/mL), and p-MLC levels were analyzed by immunoblotting 60 min after CTRP1 treatment (**top**). Quantification of the p-MLC/MLC (myosin light chain) ratio is shown (**bottom**). ***P*<0.01, Tukey test. **G**, WT and CTRP1 TG (transgenic) mice were orally administered with Y-27362 (25 mg/kg), and blood pressure (BP) was measured using the tail-cuff method at the indicated times (n=5–6 per group). ***P*<0.01, between 0 h and indicated hours after Y-27632 administration in CTRP1 TG mice, Dunnett test. Mice or rats were age matched for the in vivo study. All values are presented as mean±SD. DBP indicates diastolic BP; N.S., no significant difference between the groups indicated by the brackets; and SBP, systolic BP.

examined in the mouse aorta cell line, MOVAS. CTRP1 treatment increased MLC phosphorylation (active form) without any effect on MLCK phosphorylation (active form), whereas it increased MYPT (myosin phosphate target) phosphorylation (inactive form; Figure 5C). These effects of CTRP1 on MYPT1 and MLC phosphorylation were dose dependent (Figure 5D), and furthermore, CTRP1$^{\Delta CAG}$ mice showed a decrease in the basal levels of MYPT1 and MLC phosphorylation (Figure 5E), indicating that CTRP1-induced MYPT1 inactivation contributes to MLC activation. Next, we examined whether Rho/ROCK-signaling pathway, which is an upstream kinase pathway of MLC, could affect CTRP1-induced BP upregulation. The ROCK inhibitor Y-27632 and thiazovivin treatment completely blocked CTRP1-induced MLC phosphorylation in MOVAS (Figure 5F; Online Figure IX), and oral administration of Y-27632 effectively decreased SBP without a significant change in DBP in CTRP1 TG mice, with BP returning to hypertensive state 24 hours after medication (Figure 5G). Collectively, these findings demonstrate that CTRP1 upregulates BP via Rho/ROCK-signaling pathway–induced vasoconstriction.

Given that CTRP1 mainly targets the aorta, next, we generated vascular smooth muscle-specific CTRP1 conditional KO mice (CTRP1$^{\Delta sm22\alpha}$; Online Figure XA). CTRP1$^{\Delta sm22\alpha}$ mice were normotensive and did not show any changes in circulating CTRP1 levels (Online Figure XB and XC), whereas CTRP1$^{\Delta CAG}$ mice showed a drop in BP along with a decrease in circulating CTRP1. This data imply that CTRP1 increases BP in an endocrine manner.

## CTRP1 Stimulates the Rho/ROCK-Signaling Pathway by Enhancing Trafficking of the AT1R to the Plasma Membrane

Because Ang II activates the Rho/ROCK-signaling pathway, and we have previously shown that the CTRP1-signaling pathway is partially blocked by the AT1R blocker losartan,[17] we examined whether losartan inhibits the CTRP1-induced Rho/ROCK-signaling pathway. CTRP1-induced MYPT1 and MLC phosphorylation was effectively blocked by losartan in MOVAS (Figure 6A). Losartan treatment also decreased BP in CTRP1 TG mice, but BP completely and rapidly rebounded after losartan treatment was stopped (Figure 6B). Furthermore, Ang II-infused CTRP1$^{\Delta CAG}$ mice were less sensitive to Ang II-mediated BP increase compared with WT mice, and BP in CTRP1 TG mice was not significantly altered by continuous Ang II infusion because of enforcedly produced CTRP1 (Figure 6C), indicating that signaling through AT1R is an important effector mechanism in CTRP1-mediated vasoconstriction and the resulting BP increase. To further dissect the molecular mechanism, the interaction between CTRP1 and AT1R was assessed by cross-linking and coimmunoprecipitation, but no physical interaction between CTRP1 and AT1R was observed (Online Figure XIA and XIB). The mRNA levels of AT1Rα and AT1Rβ were also unchanged in aortas isolated from CTRP1 TG mice (Online Figure XIC through XIE). Next, we assessed whether CTRP1 stimulates trafficking of AT1R to the plasma membrane because receptor movement to the plasma membrane is able to sensitize signaling pathways. Live MOVAS imaging revealed that the EGFP-AT1R trafficked to the plasma membrane 5 minutes after CTRP1 treatment and then returned to the cytosol (Figure 6D). An immunoblot assay also showed CTRP1-induced AT1R plasma membrane trafficking (Figure 6E). To examine whether AT1R movement affects CTRP1-induced Rho/ROCK activation, EGFP-AT1R–transfected MOVAS were pretreated with the Golgi apparatus disruptor brefeldin A. Brefeldin A pretreatment completely interrupted CTRP1-induced AT1R membrane trafficking (Figure 6F), as well as CTRP1-induced MYPT1 and MLC phosphorylation (Figure 6G). Furthermore, CTRP1 TG mice showed significantly increased membranous AT1R in aortas compared with WT mice, and 2-hour oral administration of Y-27632 reduced membranous AT1R in CTRP1 TG mice to level of WT mice (Figure 6H). On the contrary, administration of Y-27632 decreased the membranous $Na^+/K^+$ ATPase, and this is probably because Rho activation stimulates the membrane trafficking of $Na^+/K^+$ ATPase.[33,34] These results imply that CTRP1 activates Rho/ROCK-signaling pathway by enhancing AT1R membrane trafficking, leading to increase in BP.

## CTRP1 Enhances AT1R Plasma Membrane Trafficking Through Phosphorylation of AKT and AS160

Next, we asked how CTRP1 stimulates AT1R plasma membrane trafficking. Given that CTRP1 stimulates GLUT (glucose transporter) 4 movement to the plasma membrane through activation of AKT-signaling pathway in C2C12 myotubes,[16] we first examined whether CTRP1 is able to activate AKT substrate of 160 kDa (AS160) via phosphorylation in MOVAS. CTRP1 treatment increased phosphorylation of AKT at T308 and S437 and phosphorylation of AS160 at T642 and S588 without altering the total levels of these 2 signaling proteins (Figure 7A). Furthermore, treatment with an AKT inhibitor reduced not only CTRP1-induced AKT and AS160 phosphorylation but also MYPT1 and MLC phosphorylation in a dose-dependent manner (Figure 7B). Moreover, AKT inhibition remarkably reduced CTRP1-induced AT1R plasma membrane trafficking (Figure 7C). Consistent with those findings, CTRP1-induced phosphorylation of MYPT1 and MLC was significantly decreased in AS160-depleted MOVAS compared with MOVAS transfected with a control siRNA (Figure 7D). Furthermore, live cell imaging (Figure 7E) and an immunoblot assay of plasma membrane fractions (Figure 7F) showed that depletion of AS160 attenuated CTRP1-induced AT1R plasma membrane trafficking in MOVAS. In addition to the results from in vitro assay, aortas isolated from CTRP1 TG mice showed an increase in the phosphorylation of AKT and AS160, as well as in the phosphorylation of MYPT1 and MLC (Figure 7G). These in vitro and in vivo results clearly support the fact that CTRP1-induced AT1R membrane trafficking through AS160 is the key event that occurs to activate Rho/ROCK downstream signaling leading to vasoconstriction. On the contrary, inhibition of ERK (extracellular signal-related kinase) and cAMP,[30] which are known to be signaling molecules that can crosstalk with AT1R and regulate its activity, was not associated with CTRP1-induced AT1R translocation to the cell surface (Online Figure XII).

## Discussion

*Ctrp1* gene could be considered as a hypotension-resistant gene because CTRP1 increased BP against dehydration-induced hypotension. Interestingly, phylogenetic tree

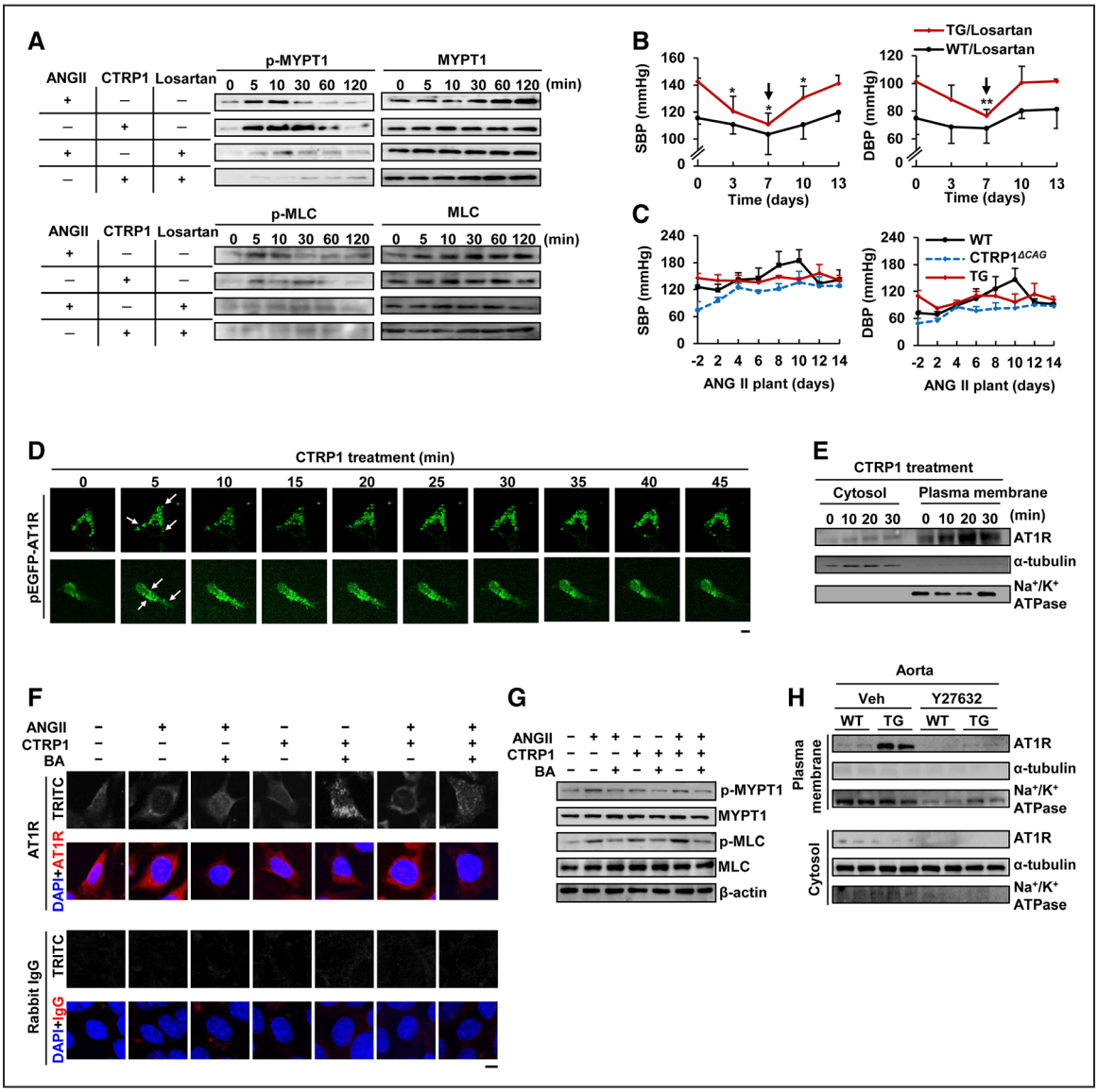


**Figure 6. The CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1)-activated Rho (Ras homolog gene family)/ROCK (Rho kinase)-signaling pathway is associated with AT1R (Ang II [angiotensin II] receptor 1) translocation. A**, MOVAS were pretreated with losartan (100 μM) 30 min before Ang II (500 nmol/L) or CTRP1 (1 μg/mL) treatment for the indicated times. The levels of the indicated proteins were examined by immunoblotting. **B**, CTRP1 TG (transgenic) and WT (wild type) mice were orally administered with losartan (1 mg/mL) for 7 d, after which blood pressure (BP) measurements were determined using the tail-cuff method (n=8–10 per group). The arrowheads indicate the day on which losartan administration was stopped. **P*<0.05, ***P*<0.01 between 0 d and indicated days after losartan administration in CTRP1 TG mice, Dunnett test. **C**, WT, CTRP1$^{\Delta CAG}$, and CTRP1 TG mice were implanted subcutaneously with osmotic pumps containing Ang II (1 μg/kg per min), and BP was determined using the tail-cuff method for 14 d (n=4 per group). **D**, MOVAS were transfected with pEGFP-AT1R and EGFP-expressing cells were monitored after CTRP1 treatment (1 μg/mL) using live imaging microscopy. The arrows indicate the expression of AT1R on cell surfaces or long membrane process (scale bar=5 μm). **E**, MOVAS were treated with CTRP1 (1 μg/mL), after which the cells were fractionated and the cytosol and plasma membrane were isolated. The expression levels of AT1R were examined by immunoblotting. The protein expression levels of α-tubulin and $Na^+/K^+$ ATPase were analyzed as loading controls for the cytosolic and plasma membrane fractions, respectively. **F** and **G**, MOVAS were pretreated with brefeldin A (10 μg/mL) 30 min before a 20-min treatment with Ang II (500 nmol/L) or CTRP1 (1 μg/mL). The cells were immunostained for AT1R (**F**, **top**) or rabbit IgG (**F**, **bottom**) to determine its localization (scale bar=5 μm), and the levels of the indicated protein were analyzed by immunoblotting (**G**). **H**, WT and CTRP1 TG mice were orally administered with Y-27362 (25 mg/kg; n=4) for 2 h, after which cytosolic and membranous AT1R were examined in aortas by immunoblotting. The protein expression levels of α-tubulin and $Na^+/K^+$ ATPase were analyzed as loading controls for the cytosolic and plasma membrane fractions, respectively. 4′,6-diamidino-2-phenylindole (DAPI) was used to counterstain DNA for immunofluorescence, and β-actin protein levels were measured as a loading control for immunoblotting. Mice were age matched for in vivo study. All values are presented as mean±SD. BA indicates brefeldin A; DBP, diastolic blood pressure; and SBP, systolic blood pressure.

shows that *Ctrp1* genes arise at the time of the emergence of vertebrates and are conserved in Euteleostomi (Online Figure XIIIA). In particular, human *Ctrp1* gene is more homologous to terrestrial than aquatic vertebrates (Online Figure XIIIB). In the transition from aquatic to terrestrial life, vertebrates become exposed to the hot, dry, and windy environments in which conditions vertebrates should control body temperature by sweating; however, continuous and excessive dehydration by sweating can cause the loss of body fluid and salts leading to hypotension.[35,36] Thus, we deduce that *Ctrp1* gene is likely to be evolved to prevent dehydration-induced hypotension.

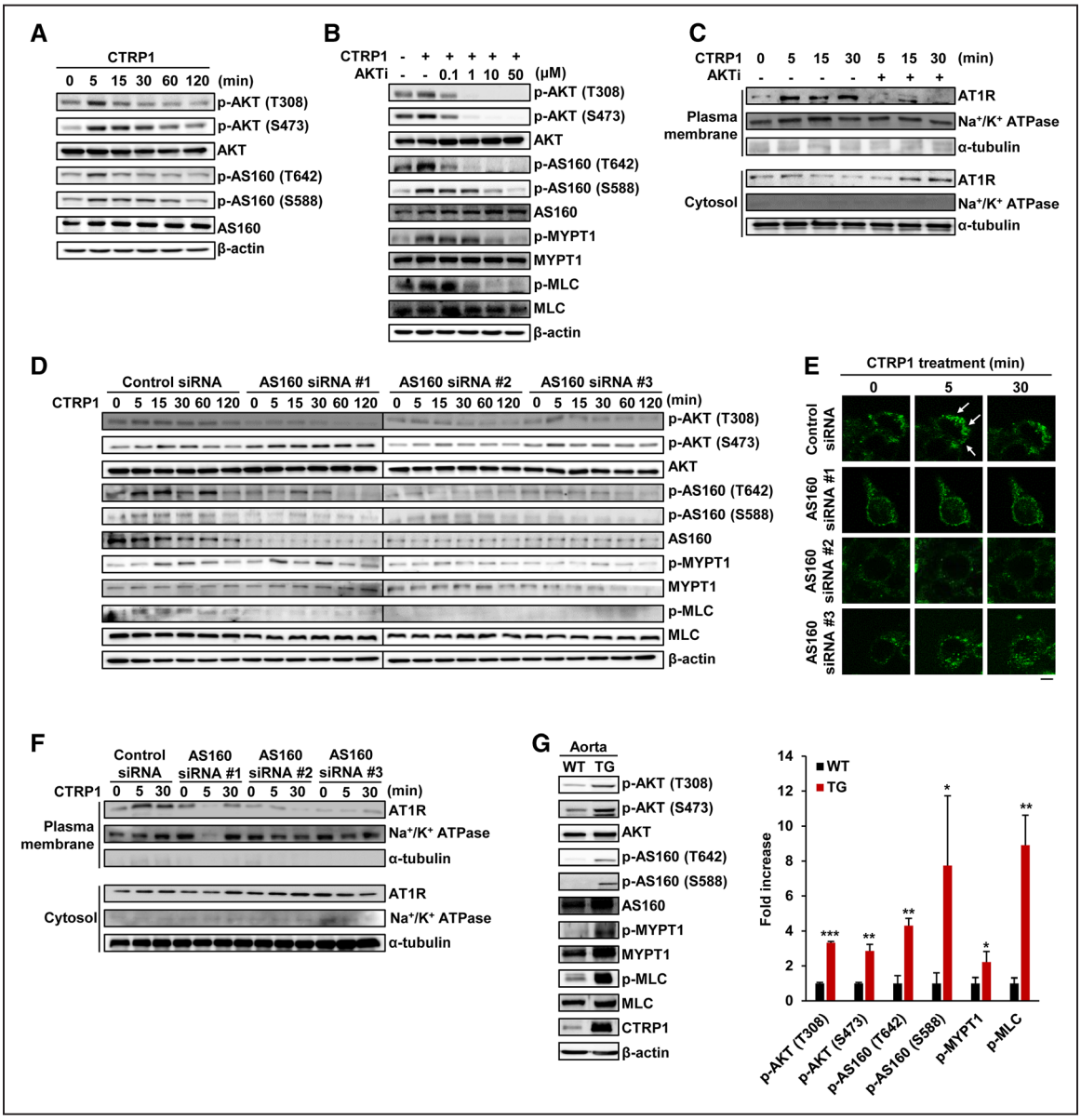


**Figure 7. CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) induces AT1R (Ang II [angiotensin II] receptor 1) translocation through the AKT/AS160-signaling pathway. A**, The levels of phosphorylated AKT and AS160 in MOVAS were analyzed using immunoblotting after CTRP1 treatment (1 μg/mL) for the indicated times. The β-actin protein levels were analyzed as a loading control. **B**, MOVAS were pretreated with the indicated concentrations of AKTi (AKT inhibitor) 30 min before a 5-min treatment with CTRP1 (1 μg/mL), and the levels of the indicated protein/phosphoproteins were analyzed by immunoblotting. β-actin protein levels were analyzed as a loading control. **C**, MOVAS were pretreated with AKTi (10 μM) 30 min before treatment of CTRP1 (1 μg/mL) for the indicated times, and cytosolic and plasma membrane fractions were isolated. The expression levels of the AT1R were then examined by immunoblotting. α-tubulin and $Na^+/K^+$ ATPase protein levels were analyzed as loading controls for the cytosolic and plasma membrane fractions, respectively. **D–F**, Three small interfering RNAs (siRNAs) against different regions of AS160 were designed and MOVAS were transfected with these AS160 siRNAs or a control siRNA (20 nmol/L) for 48 h. **D**, Levels of the indicated proteins/phosphoproteins in MOVAS were analyzed by immunoblotting in the siRNA-transfected cells after CTRP1 treatment (1 μg/mL) for the indicated times. β-actin protein levels were analyzed as a loading control. **E**, MOVAS cells transfected with the 3 siRNAs were transfected with pEGFP-AT1R, and cells harboring GFP (green fluorescent protein) were monitored at the indicated times after CTRP1 treatment (1 μg/mL) using live imaging microscopy. The arrows indicate expression of AT1R on the cell surface (scale bar=5 μm). **F**, The siRNA-transfected cells were treated with CTRP1 (1 μg/mL), and cytosolic and plasma membrane fractions were isolated, after which the expression levels of AT1R were examined by immunoblotting. α-tubulin and $Na^+/K^+$ ATPase protein levels were analyzed as loading controls for the cytosolic and plasma membrane fraction, respectively. **G**, The expression levels of the indicated phosphoproteins were examined in aortas from WT (wild type) and CTRP1 TG (transgenic) mice by immunoblotting (**left**), and their ratio to total protein levels were quantified (**right**; n=3 per group). The β-actin protein levels were analyzed as a loading control. All mice were age matched for in vivo study. Values are presented as mean±SD. **P*<0.05, ***P*<0.01, ****P*<0.001 between CTRP1 TG and WT mice, 2-tailed Student *t* test.

Decrease in BP in response to dehydration can trigger signaling processes to restore BP, which is exerted through 2 temporal mechanisms. One is a short-term mechanism, which regulates blood vessel diameter, heart rate, and contractility. This short-term regulation is exerted by sympathetic nervous system, which secretes epinephrine/norepinephrine from the adrenal gland.[29] The other is a long-term mechanism, which is accomplished by the renal-dependent renin-Ang II (angiotensin II) system activity and renal-independent glucocorticoid secretion.[29] In the present study, we provide evidence that

CTRP1 acts as a novel long-term BP regulator through the following experimental evidence: (1) dehydration increases CTRP1 production, (2) a synthetic glucocorticoid directly increases CTRP1 production, (3) a glucocorticoid antagonist reduces dehydration-induced CTRP1 levels, (4) CTRP1$^{\Delta CAG}$ mice are not able to maintain BP under dehydration conditions, and (5) CTRP1 production is increased 2 days after mice are placed under dehydration conditions. Attenuated BP increase in electric foot shock-received CTRP1$^{\Delta CAG}$ mice supports that CTRP1 is a key effector of glucocorticoid-mediated BP regulation. This result also implies that CTRP1 can regulate BP under other conditions that increase glucocorticoid. Nonetheless of glucocorticoid secretion is stimulated by sympathetic nervous system, CTRP1 is likely to be regulated by hormonal stimulation because glucocorticoid increases CTRP1 at long term.

It is thought that skeletal muscle is passively involved in BP regulation by mechanical blood vessel contraction.[37] However, here, we provide evidence that muscle is actively engaged in BP regulation through CTRP1 production in response to dehydration and glucocorticoid secretion. Thus, CTRP1 could be classified as a novel myokine along with CTRP15, which is specifically expressed in the skeletal muscle and participates in lipid metabolism and autophagy.[38] Moreover, CTRP1$^{\Delta sm22\alpha}$ mice showed an intact circulating CTRP1 level, indicating that a major source of circulating CTRP1 is the skeletal muscle. On the contrary, circulating cortisol levels were not significantly altered in hypertensive patients compared with normotensive individuals and also did not show correlation with CTRP1 (Online Figure XIVA and XIVB). We speculate that this contradictory result might be because of the inconsistency between local and systemic concentration of cortisol. CTRP1 could be increased by local cortisol within skeletal, heart, and aorta muscles instead of systemic cortisol because myotubes express 11-β hydroxysteroid dehydrogenase, which converts biologically inert cortisone to biologically active cortisol.[39]

Dehydrated CTRP1$^{\Delta CAG}$ mice and ROCK inhibitor-treated CTRP1 TG mice showed more sensitive changes in SBP than in DBP. Given that CTRP1 acts as a vasoconstrictor, a sensitive change in SBP change is logical because SBP is more responsive to vasoconstrictors than DBP.[40,41] On the contrary, it is unlikely that the CTRP1-mediated BP increase is associated with endothelium-dependent vasorelaxation not only because CTRP1 was poorly expressed in epithelial cells but also because endothelium-denuded aortic rings isolated from CTRP1$^{\Delta CAG}$ KO mice were less contracted by Ang II compared with those from WT mice, although aortic rings from mice poorly response to Ang II compared with aortic rings from rats[42] (Online Figure XVA and XVB).

Rho/ROCK negatively regulates eNOS (endothelial NO synthase)/NO signaling.[43] However, CTRP1 is likely to induce vasoconstriction through eNOS/NO-independent mechanism because CTRP1 treatment did not significantly alter the levels of NO, eNOS, and phosphorylation of eNOS at S1177 in aortic rings isolated from WT mice (Online Figure XVC and XVD). In addition, either CTRP1 deficiency or excess did not affect the eNOS mRNA levels in the aortas (Online Figure XVE), and no significant alterations of total and phosphorylated eNOS were observed in those mice under dehydration conditions (Online Figure XVF). Furthermore, CTRP1 did not affect the production of NO in MOVAS cells (Online Figure XVG).

In this study, we found that CTRP1 increases MYPT1 phosphorylation through activation of the Rho/ROCK-signaling pathway and that CTRP1-induced Rho/ROCK activation depends at least on AT1R. In aortas, membranous AT1R and cytosolic MLC phosphorylation were increased under dehydration condition and returned to normal levels by rehydration, which are similar to the fluctuation of CTRP1 level under water restriction conditions, indicating that CTRP1, plasma membrane trafficking of AT1R, and MLC phosphorylation closely cross talk each other to regulate BP under dehydration conditions (Online Figure XVI). The attenuated BP upregulation in Ang II-implanted CTRP1$^{\Delta CAG}$ mice also supports the fact that CTRP1 is required for activation of the Ang II/AT1R-signaling pathway, even though further studies are required to elucidate the fine molecular mechanisms by which CTRP1 and Ang II cooperatively or independently regulate BP. Because the receptor for CTRP1 has not yet been identified, we hypothesize that CTRP1-stimulated AT1R plasma membrane trafficking itself could sensitize AT1R, which is easily activated by a small amount of Ang II.

Nonetheless, we provide a mechanism by which CTRP1 stimulates AT1R plasma membrane trafficking. AS160 was identified as an AKT substrate in the adipose tissue and contains a Rab-GAP (GTPase-activating protein) domain at its COOH terminus and multiple AKT phosphorylation sites.[44] Phosphorylation of these sites by AKT stimulates GLUT4 translocation toward the cell membrane in adipocytes and myocytes in response to insulin.[44,45] Newly identified evidence has shown that AKT/AS160 signaling regulates the plasma membrane trafficking of various receptors in various tissues and cell types, including GLUT1,[46,47] GLUT8,[48,49] aquaporin 2,[50,51] β1-integrin,[52] CD36 (cluster of differentiation 36),[53,54] epithelial $Na^+$ channel,[55] potassium voltage-gated channel Kv1.5,[56] and $Na^+/K^+$ ATPase.[57,58] In this study, we show for the first time that AS160 is activated by the CTRP1-AKT axis, leading to AT1R plasma membrane trafficking. This new finding is supported by the evidence that treatment with an AKT inhibitor, or knockdown of AS160 in MOVAS, markedly inhibits CTRP1-induced Rho/ROCK activation associated with attenuated AT1R plasma membrane trafficking. In addition, CTRP1 TG mice showed high levels of phosphorylated AKT and AS160 in the aorta, and we have previously shown that CTRP1 increases GLUT4 translocation to the plasma membrane through AKT phosphorylation at S473.[16] On the contrary, because multitargeted tyrosine kinase inhibitors sorafenib and sunitinib effectively suppressed CTRP1-induced phosphorylation of AKT, AS160, MYPT1, and MLC, we suggest that CTRP1 transmits the signal into AKT through a tyrosine kinase (Online Figure XVII).

The translocation of RhoA and PKCα (protein kinase Cα) to the plasma membrane positively regulates the vasoconstriction in response to Ang II.[59] Interestingly, CTRP1 increased the plasma membrane trafficking of RhoA and PKCα (Online Figure XVIIIA and XVIIIB) and increased

the phosphorylation of PKCα at S657, which is an indication of PKCα activation[60] (Online Figure XVIIIC). Moreover, pharmacological PKCα inhibition abolished CTRP1-induced p-MYPT1, p-MLC, p-AKT at S473, and p-AS160 at S588 (Online Figure XVIIID). These results show that PKCα is also involved in CTRP1-induced activation of Rho/ROCK-signaling pathway although marginal alteration was observed in aortas from CTRP1$^{\Delta CAG}$, WT, and TG mice (Online Figure XVIIIE). On the contrary, it is also possible that hypertensive phenotype of CTPR1 TG mice is affected by changes in cytokines, chemokine, and growth factors. CTRP1 TG mice showed a significant increase in circulating G-CSF (granulocyte-colony stimulating factor), active IL-12 (interleukin-12) heterodimer (IL-12 P40/p70), MCP-1 (monocyte chemoattractant protein 1), and TNFR1 (tumor necrosis factor receptor 1) without changes in circulating and transcription levels of ET-1 (endothelin-1) in aortas, which is well-known vasoconstrictor,[61] indicating that CTPR1-mediated BP increase is ET-1 independent, but role of cytokines increased in CTRP1 TG mice remains to be studied (Online Figure XIXA through XIXC).

We showed that CTRP1 is increased in hypertensive patients, implying the pathophysiological role of CTRP1 in hypertension. Aged CTRP1 TG mice also showed similar histological impairments to hypertensive patients who show the left ventricular hypertrophy and renal structure failure,[62] suggesting that abnormal production of CTRP1 is a novel pathogenesis for hypertension. Furthermore, clinical data showed that circulating CTRP1 levels are not related to sex although they were positively correlated with age (Online Figure XXA and XXB). In addition, circulating CTRP1 levels in hypertensive patients medicated with calcium channel blockers or AT1R blockers were similar with those in normotensive individuals (Online Figure XXI). To obtain a more comprehensive conclusion, CTRP1 and BP measurements before and after medication in a newly diagnosed hypertensive patient are absolutely needed. Nonetheless, the limitation of this clinical data analysis was that CTRP1 values were not normalized by diuretic medication, circulating glucocorticoid levels, or blood volume status, and this should be addressed in a future study.

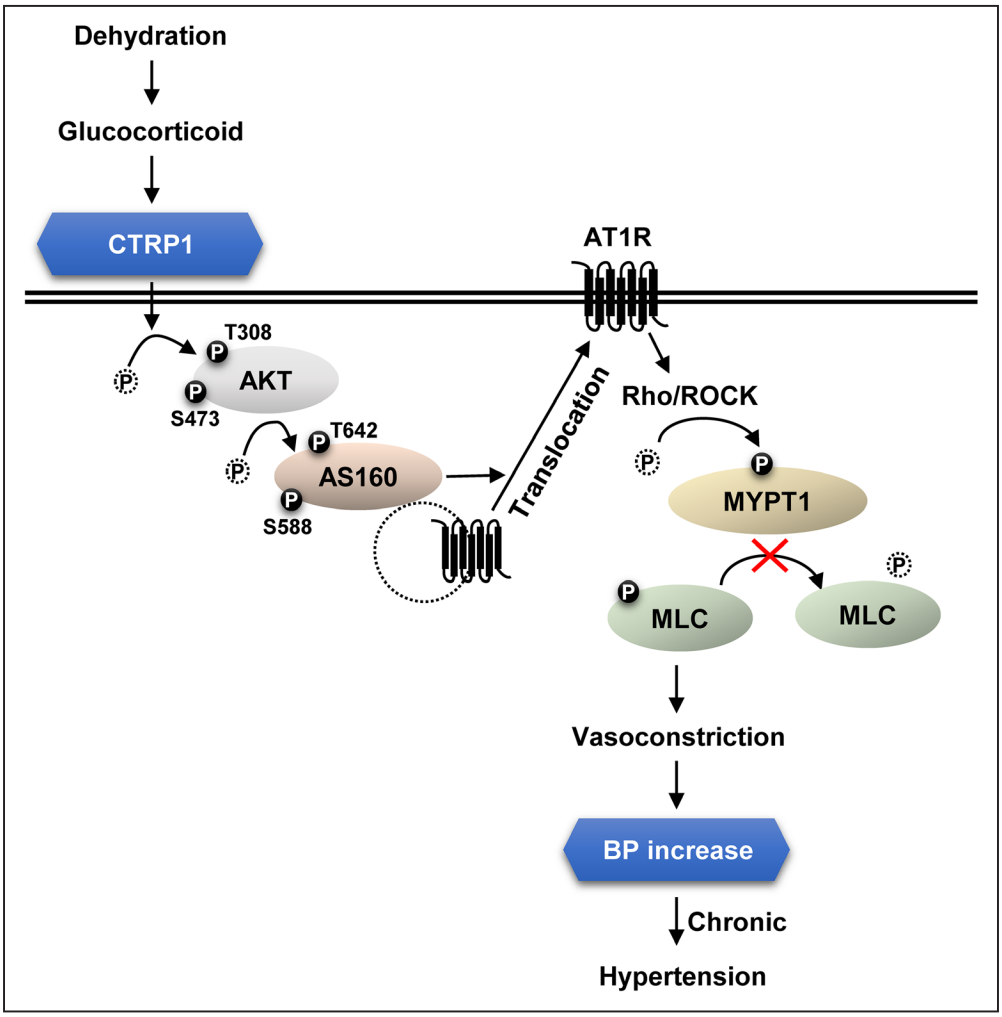


**Figure 8. Proposed role of CTRP1 (C1q/TNF-α [tumor necrosis factor-α]–related protein 1) in the blood pressure (BP)-regulatory mechanism under dehydration conditions.** Under dehydration conditions, CTRP1 increases in response to glucocorticoid. CTRP1 activates AKT by phosphorylation, and AKT inactivates AS160 by phosphorylation. This positively stimulates AT1R plasma membrane trafficking and its downstream Rho (Ras homolog gene family)/ROCK (Rho kinase)–signaling pathway. Inactivation of MYPT1 (myosin phosphate target subunit 1) by phosphorylation sustains MLC (myosin light chain) phosphorylation, which leads to vasoconstriction and BP increase. Therefore, the chronic state of excessive CTRP1 is positively related with the onset of hypertension.

In summary, we have identified a novel role of CTRP1 in the long-term, nonrenal-mediated upregulation of BP that occurs under dehydration conditions (Figure 8). In this mechanism, dehydration-induced glucocorticoid increases CTRP1, which activates AKT/AS160-mediated AT1R movement to the plasma membrane. This leads to AT1R sensitization and activation of its downstream Rho/ROCK-signaling pathway, which sustains the MLC phosphorylation leading to vasoconstriction.

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## Disclosures

None.

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